Characterizing Novel Fluorescent Protein Pairings for Förster Resonance Energy Transfer (FRET)

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Abstract

Since it's cloning, the sequence encoding the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* has been engineered to produce fluorescent proteins (FPs) with emission in the blue to yellowish-green range of the visible spectrum. Furthermore, many FPs with homology to the *Aequorea* GFP have been cloned from marine organisms, providing new proteins that fluoresce into deep red spectrum. These new FPs expanded the repertoire of applications to include multi-color imaging of protein co-localization and behavior inside living cells. However, it is their use as donor and acceptor pairs for Förster Resonance Energy Transfer (FRET) microscopy that has generated the greatest interest. The most precise methods for measuring FRET detect the quenching of the donor by the acceptor, and Fluorescence Lifetime Imaging Microscopy (FLIM) can accurately measure the shorter donor lifetimes that result from FRET. Currently, the *Aequorea* GFP variants known as Cerulean (cyan) and Venus (yellow) are the most popular FRET pair. However, Venus has poor photostability, and the emission near 500 nm limits its utility as an acceptor. The objective of this study was to use FLIM to test the utility of different FP pairings for FRET studies with the goal to identify potentially improved FRET pairs.